International Journal of Medicine
and Pharmaceutical Sciences (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 5, Issue 4, Aug 2015, 53-68

TRANS STELLAR
• Journal Publications • Research Consultancy

RICE BRAN OIL AND ORYZANOL ATTENUATES DYSLIPIDEMIA AND OXIDATIVE STRESS IN ATHEROGENIC DIET FED RATS

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ABSTRACT

Hyperlipidemia is the one of the prime factor for atherosclerosis and cardiovascular diseases. Disorders of lipid metabolism are associated with excess production of free radicals and eventually oxidative stress. The study was undertaken to assess the lipid lowering activity of rice bran oil (RBO) and oryzanol (OZ) in rats. The Male albino wistar strain was rendered hyperlipidemic by feeding atherogenic diets (15% coconut oil and 1% cholesteol). Dietary regime was followed for a period of 42 days. Serum and tissues (liver and brain) were investigated for its antihyperlipidemic and ant oxidative efficacy in rats. Atherogenic diets resulted in significant alterations in lipid – lipoprotein fractions and oxidative stress biomarkers in serum and tissues of rats. Treatment with RBO and oryzanol significantly ($p \leq 0.05$) restored the biochemical indices near to normal. Results reveal that RBO and OZ ameliorated lipid abnormalities and improved the antioxidant enzymatic status in experimentally induced hyperlipidemia in rats by virtue of antioxidative potential of unsaponifiable matter.

KEYWORDS: Rice Bran Oil, Oryzanol, Antioxidants, Dyslipidemia, Oxidative Stress, Antioxidant Defense System

INTRODUCTION

Cardio vascular diseases amongst the other degenerative diseases are the highest risk factor for morbidity and mortality around the globe. The incidence has increased alarmingly over last two decades of the 20th century with multifactorial causes, having not only genetic susceptibility but also inputs from hypertension, hypercholesterolemia, obesity, low physical fitness along with dietary, behavioral and environmental quarters. Dietary lopsidedness converge on a diet high in saturated fats, sugar, refined products and foods of animal origin with lower intake of fibre, whole grains, fruits and vegetables. These dietary changes may not influence the present health but may determine whether or not the individual will develop such diseases much later in life. There is an urgent need to switchover and rectify our dietary imbalances for better metabolic efficiencies. The dietary modifications generally act at a low pace; however the probabilities of undesirable effects are comparatively negligible as compared to the widespread pharmacotherapies.

Rice bran oil (RBO) has been used as edible oil in Japan for several decades and is popularly known as *"Heart Oil"*. In recent years, a tremendous interest has been generated in cholesterol lowering and antioxidative properties of RBO and its bioactive compounds. India ranks first in the annual production of crude RBO (500,000 tones) and refined RBO (>400,000 tones) (Gopalakrishna et al., 2006), however, the possibility of exploiting rice bran as a source of edible oil has come into greater focus in our country in recent years and the nutritional composition, toxicological safety and

hypolipidemic action of RBO have been studied with interest. The chemical composition of RBO is very close to that of groundnut oil (GNO) however, it has high unsaponifiable fraction (1.5-2.6%) in contrast to other refined vegetable oils that contains only 0.3-0.9% (Rong et al., 1997). The relatively high level of unsaponifiable components contributes to hypolipidemic effect, henceforth reduces the risks of atherosclerosis and coronary heart diseases. Epidemiological evidences have attributed, the cholesterol lowering property of RBO, to the presence of oryzanol, a unique component in the oil, which is not found in any other edible oil and also to some other components present in unsaponifiable matter (Rong et al., 1997; Seetharamaiah and Chandrasekhara, 1989; Sharma and Rukmini, 1987).

γ-Oryzanol is a mixture of ferulic acid derivatives (Yoshino et al., 1989) and has multitude pharmacological effects and has been used to decrease plasma cholesterol, cholesterol absorption from gastro intestinal tract as well as aortic fatty streaks, platelet aggregation (Hakala et al., 2002), regulation of the estrous cycle, growth-acceleration, and the ability to promote skin capillary circulation (Rong et al., 1997). In view of the above considerations the study was planned to assess the antioxidative efficacy of RBO and oryzanol in hyperlipidemic rats.

MATERIALS AND METHODS

Chemicals

All the chemicals used in the study were of analytical grade, procured from Sigma, Merck, BDH and Qualigens. Diagnostic kits for the estimation of serum and hepatic biomarkers were purchased from Erba Mannheim (Transasia Bio-Medicals Ltd. Daman, India). RBO was provided from A.P Solvex Ltd, Dhuri, India. Gamma oryzanol was purchased from Qingdao Reach International Inc, China.

Experimental Animals

The study protocol was approved by Institutional Animal Ethics Committee (IAEC) of the University constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Forty two healthy male albino wistar rats of 6 weeks age (50.2±2.86g) were procured from the small animal house of Chaudhary Charan Singh Haryana Agriculture University Hissar (CCSHAU), India. Each animal was housed individually in the polypropylene cage with sterilized wood chip bedding in a specific pathogen free animal house room under the constant environmental condition with 12 hour light and dark cycle, 22 ± 1^{0}C temperature; 50 ±10% relative humidity and artificial illumination between 06:00 and 18:00 hrs. Animals were given the standard pellet diet (Hindustan Liver Ltd.,India) and water *ad libitum* during acclimatization period of 1 week. The diet contained 20% protein, 5% protein, 5% fat and 5% fibre, 60% carbohydrates and 10% mixture of vitamins and minerals. The diet served as normal fat diet (NFD) for control group. Atherogenic diet was formulated incorporating 83.87% NFD; 15% coconut oil (w/w); 1% cholesterol (w/w) and 0.125% (w/w) taurocholate.

Acute Toxicity Studies

Acute oral toxicity was performed as per organisation for Economic Cooperation and Development (OECD) guidelines 423 (2001). After the dietary supplementation of experimental diets, animals were observed individually for general behavioural at least once during the first 30 minutes and periodically during the first 24 hours with special attention given during first four hours and daily thereafter for a period of 14 days. At the end of the experimental period, all survivors were sacrificed to examine gross changes in their vital organs.

Experimental Diets

Animals with similar average body weight were randomly divided into seven groups. The feeding regime was carried for a period of six weeks and animals were given different dietary treatments as: NC (n=6), fed NFD and served as normal control; The other treatment groups were reared on atherogenic diets (AD) comprising of high fat and high cholesterol (HFHC) as under: AD, served as experimental control fed HFHC diet ; AD-RBO; fed HFHC + RBO (15%); AD-RBO-OZ-100; fed HFHC + RBO (15%) + γ-Oryzanol (OZ) (100mg/kg body weight); AD-RBO-OZ-200; fed HFHC + RBO (15%) + γ-Oryzanol (OZ) (200mg/kg body weight); AD-OZ-100; fed HFHC + γ-Oryzanol (OZ) (100mg/kg body weight);. AD-OZ-200; fed HFHC + γ-Oryzanol (OZ) (200mg/kg body weight). Basal and experimental diets were isoenergic (~3600C) and were freshly prepared weekly in a pathogen free sterilized room (Reeves et al; 1993) and were stored at -20^{0}C.

Biochemical Assays

For biochemical parameters the animals were bled from retrorbital plexus at the end of feeding schedule, centrifuged at 3000g at 4^{0}C for 10 minutes to obtain serum. At the end of the six weeks treatment, 24-hour fecal samples were collected from individual cages for assay of neutrol steols, cholesterol and bile acids and the animals were sacrificed by cervical decapitation. The organs (liver and brain) were removed, freed of adhering tissues, washed with ice cold isotonic saline, blotted dry and weighed. A small part of the tissues were excised, minced and was used for enzyme activity assay and other biochemical evaluation. The remaining tissues were stored at -80 ^{0}C for further biochemical analysis.

Biochemical Analysis of Serum

Serum total cholesterol (TC), triglycerides (TG) and HDL-cholesterol were estimated by using diagnostic kits (Erba Mannheim Transasia Bio-Medicals Ltd. Daman, India). VLDL and LDL-cholesterol were calculated as per Friedewald's equation (1972): VLDL-C = TG/5 and LDL-C =TC – (HDL-C + VLDL-C). Serum indices were calculated as: TC/PL; HDL-C/TC; HDL-C/ Non HDL-C and Atherogenic index (AI) = (Total-C - HDL-C)/HDL-C. Hepatic and faecal lipids were extracted by method of Folch et al., (1957). HMG Co.A reductase activity was assayed by the method of Rao and Ramakrishnan (1975) and expressed as the ratio of absorbance of HMG- CoA to mevalonate. Hepatic bile acid was estimated by the method of Snell and Snell (1953). Faecal matter was analysed for bile acid (Snell and Snell, 1953), neutral sterols (Kalek et al., 1984) and cholesterol using diagnostic kits (Erba Mannheim Transasia Bio-Medicals Ltd. Daman, India). Serum glutamate pyruvate transaminases (ALT) and serum glutamate oxaloacetate transaminases (AST) were measured spectrophotometrically by utilizing the method of Reitman and Frankel (1979). Gamma glutamyl transferase activity was measured by the Rosalki and Rau Method (1972).

Biochemical Analysis of Tissue Homogenates

For enzyme activity assay, 0.8-1.0g of tissue (liver) was minced and homogenized in 10 times its volume of 0.2M/L tris HCl (pH=8.0) containing 0.5M/L $CaCl_2$ using Potter Elevehjem apparatus at 0-4^{0}C using motor driven Teflon pestle rotated at 3000rpm. The homogenate was centrifuged at 10000g for 30 minutes at 4^{0}C and 3/4th of the volume was carefully drawn using Pasteur's pipette. The supernatants were stored at -80^{0}C until analysis. Brain is a vulnerable organ for oxidative damage due to high levels of oxygen uptake and utilization, high lipid content and low antioxidant capacity. Rat skull was opened carefully and the brain without any mincing was washed with ice cold saline (0.9%), weighed and homogenized at 4^{0}C in 0.15M KCl (200mg/10ml), centrifuged at 10,000xg for 15 minutes and stored at -80^{0}C until

analysis. Enzyme assay involved, lipid peroxidation (TBARS) (Ohkawa et al., 1979), reduced glutathione (GSH) (Sedlak & Lindsey, 1968) and antioxidant enzymes viz. glutathione peroxidase (GSHPx) (Necheles et al., 1968); catalase (CAT) (Luck, 1971); superoxide dismutase (SOD) (Kono, 1978); glutathione -S-transferase (GST) (Habig et al.1974) and protein carbonyl (PCO) (Reznick and Packer, 1994; Levine et al., 1990).The protein content was estimated using Bio-Rad protein assay kit and BSA as standard.

Statistical Analysis

Results are expressed as Mean ± Standard Error of Mean (SEM) of 6 rats. Statistical analysis involved Analysis of Variance (ANOVA- one-way). Tukey's post-hoc multiple comparison test was carried out using SPSS (version 16.0) and student's't'-test using Sigma Plot (version 8.0). The values at $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSIONS

Effect on Growth Parameters

Animals fed on atherogenic diet (AD) showed a significant increase ($p \leq 0.05$) in body weight and relative food consumption (RFC). The effect was nullified for both the parameters by supplementation of RBO and OZ to a marked extent, among the treatment groups; however the variation in between the treated groups was not significant ($p \geq 0.05$) and were within the range of (68.4% - 70.8%) and (6.9±1.24 - 7.2±1.21g/100g b.w.). Relative liver size (RLS) increased markedly in AD group (155.4±2.48 g/100g b.w) as compared to NC group (130.9±2.54g/100g b.w). The animals treated with RBO and OZ enriched diets tended to neutralize the effect, while the most pronounced effect was observed in AD-OZ-200 group (135.2±2.14 g/100g b.w) followed by AD-RBO-OZ-200 (139.7±2.64 g/100g b.w) as evident from Table 1. An upsurge in adipose tissue was observed in AD group in response to high fat and high cholesterol feeding. Supplemented diets reversed the effect and restored the levels to near normal. High fat feeding results in hyperphagia and induces hyperlipidemia resulting in proliferation, differentiation of adipocytes leading to hepatomegaly and accumulation of excess fats in adipose tissue. The results are in accordance with previous studies whereby RBO and its bioactive compounds resulted in reduction of organ and body weights by preventing the accumulation of lipids (Wang et al., 2014; Ha et al., 2005).

Table 1: Effect of Atherogenic Diets on Growth Parameters and Adipose Tissue in Hyperlipidemic Rats

Treatment	Weight Gain	RFC	RLS	Adipose Tissue		
	%	(g/100g Body wt)	(g/100g Body wt)	Epidydmus g	Perirenal g	Mesentric G
NC	71.7	6.7±1.01	130.9±2.54	4.5±0.12	0.58±0.01	2.1±0.002
AD	76.8[a]	7.5±1.53 [a]	155.4±2.48 [a]	6.7±0.15 [a]	1.1±0.01 [a]	3.3±0.03 [a]
AD-RBO	70.8[b]	7.1±1.41 [b]	145.8±2.84 [b]	4.3±0.25 [b]	0.78±0.02 [b]	2.7±0.04 [b]
AD-RBO-OZ-100	69.3 [b]	7.2±1.21 [b]	143.5±2.75 [b]	4.7±0.43 [b]	0.76±0.04 [b]	2.6±0.01 [b]
AD-RBO-OZ-200	68.4 [b]	7.1±1.05 [b]	139.7±2.64 [b]	4.6±0.51 [b]	0.64±0.03 [b]	2.4±0.01 [b]
AD-OZ-100	70.8 [b]	7.1±1.34 [b]	146.2±2.87 [b]	4.4±0.87 [b]	0.66±0.01 [b]	2.5±0.04 [b]
AD-OZ-200	69.6 [b]	6.9±1.24 [b]	135.2±2.14 [b]	4.3±0.46 [b]	0.61±0.04 [b]	2.3±0.03 [b]

Values are Mean ± SEMof 6 rats in each group
Group NC is compared with Groups AD
Other Treatment groups are compared with AD
[a]$p \leq 0.05$: Significantly different from NC
[b]$p \leq 0.05$: Significantly different from AD
NS: Non Significant

Serum Lipid- Lipoprotein Fractions

Dyslipidemia comprising of hyperlipidemia, hypercholesterolemia and hypertriglyceridemia is the major risk factor in the onset and progression of cardiovascular diseases (CVD). Several clinical trials have indicated that the alterations in lipid- lipoprotein fractions (decreased LDL-C and VLDL-C with an increase in HDL-C levels) either by dietary or pharmacological means reduces the incidence of mortality due to CVD (Shepherd et al., 1995; Sacks et al., 1996); henceforth the focus of research for the past decades have been shifted to quality of dietary fat independent to the quantity of total amount (Grundy and Denke, 1990; Chou et al., 2009).

Figure1 (A) depicts that serum lipid-lipoprotein fractions were markedly altered in AD group. A significant increase ($p \leq 0.05$) was observed in TC; TG; PL; LDL-C and VLDL-C with marked decrease in HDL-C levels indicative of dietary induced hyperlipidemia and probably an increased risk to cardiovascular diseases. On the contrary the altered lipid parameters were restored to near normal levels in RBO and OZ treated groups. Of lipoprotein fractions, HDL-C significantly increased ($p \leq 0.05$) and a notable effect was observed in rats fed on AD-OZ-200 (42.1±1.27mg/dl) followed by AD-OZ-100 (40.2±0.91mg/dl). HDL reduces the amount of deposited cholesterol in endothelium by retrieving cholesterol from peripheral cells and other lipoproteins to the liver for excretion in bile, thereby reducing the risk of CVD. (Wang et al., 2014; Zou et al., 2005; Martinez et al., 2004).

The maximal reduction in LDL-C was in AD-OZ-200 (12.8±0.63mg/dl) followed by AD-RBO-OZ-200 (14.8±0.84mg/dl). Improvement in LDL- C levels may be due to the activation of LDL receptors or inhibiting cholesterol biosynthesis, consistent with previous studies (Ahmad et al., 2013; Chauhan et al., 2010; Chou et al., 2009;) VLDL-C levels showed marked improvement in the treated groups in contrast to AD group; however, significant ($p \geq 0.05$)variation between the treated groups was not seen. Wilson et al., (2007) reported that at equal dietary intake of oryzanol and ferulic acid, oryzanol has greater effect on lowering plasma non HDL-C levels and raising HDL-C possibly by faecal excretion of cholesterol and its metabolites, thus confirming to other studies (Sharma and Rukmini, 1986; Seetharamaiah and Chandrasekhara, 1989). Apo A levels decreased with a simultaneously with an increase in Apo B and Lp (a) levels in AD group as compared to normal rats. In contrast improvement was observed in the indices of treated animals, though it was not significant ($p \geq 0.05$). It is evident from the Figure 1 (A) and (B); the maximal increase in HDL-C (42.1±1.27mg/dl) and Apo A (0.029±0.001g/L) levels were observed in AD-OZ-200 group. Lipoprotein (a), also called Lp(a), is a lipoprotein subclass; it is a “suis generis” molecule consisting of a low-density lipoprotein (LDL)-like particle whose apoB-100 is covalently bound to the apoprotein(a) (apo(a)) through a disulfide bridge (Uccello et al., 2011). There is a strong correlation between elevated Lp(a) and heart disease that has led to the consensus that Lp(a) is an important, independent predictor of cardiovascular disease (Nordestgaard et al., 2010). Animal studies have shown that Lp(a) may directly contribute to atherosclerotic damage by increasing plaque size, inflammation, stability, and smooth muscle cell growth (Kamstrup & Nordestgaard, 2009)

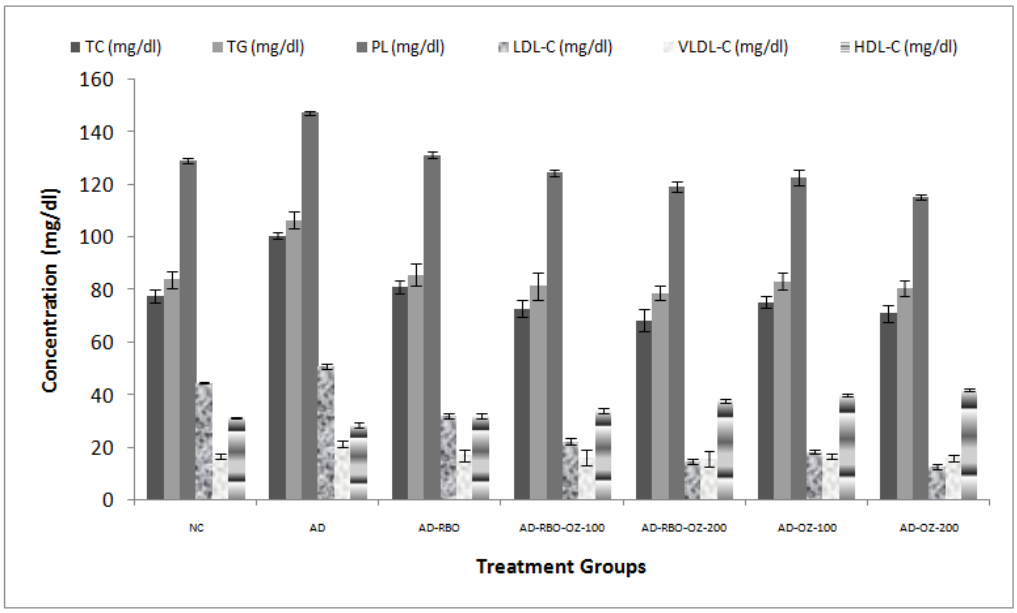

Figure 1: (A) Effect of RBO and OZ Enriched Atherogenic Diets on Serum Lipid-Lipoprotein Fractions in Hyperlipidemic Rats (Values (Mean± SEM) of 6 Rats in Each Group)

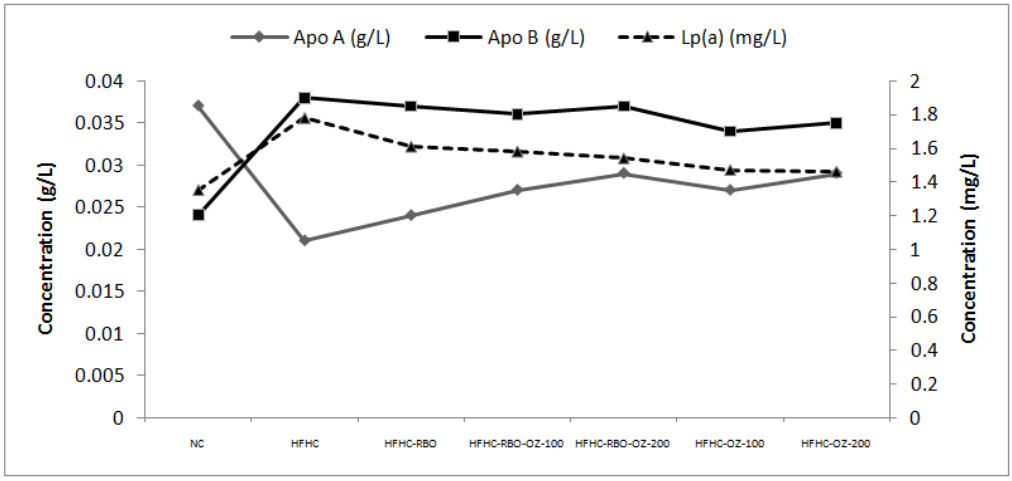

Figure 1: (B) Effect of RBO and OZ Enriched Atherogenic Diets on Serum Apo A; Apo B and Lp (a) Levels in Hyperlipidemic Rats (Values (Mean± SEM) of 6 Rats in Each Group)

Serum Indices

The serum indices showed a marked improvement in the treated animals in contrast to the AD fed animals. Figure2 indicate a significant increase ($p \leq 0.05$) in mean HDL-C/Total-C; HDL-C/Non HDL-C and a significant decrease ($p \leq 0.05$) in mean TC/PL and atherogenic index (AI) of treated animals, thus, positively affecting the lipoprotein status of the experimental animals. A similar finding was observed in a study which demonstrated that serum indices increased in rats with the feeding of rice bran oil at 5% and 20% levels as compared to the peanut oil fed at the same levels (Purushothma et al., 1995). Marsch et al., (1976), reported that increased concentration of cholesterol can result in a relatively large increase in molecular ordering of residual phospholipids resulting in a decrease in membrane fluidity. Narasimhamurthy et al., (1997) have demonstrated that in healthy conditions, the ratio of TC/PL in plasma is ≤1.0 while in hypercholesterolemia it is >1.0. The results of the present study revealed that the ratio of TC/PL in atherogenic diet fed animals increased as compared to the control probably due to increased serum total cholesterol. However, the mean values

remained within normal range (≤1.0) due to concomitant variations in the two components. RBO and OZ enriched group caused a further simultaneous decrease in both the components suggestive of their hypocholesterolemic action. Sterol content of the membrane is an important determinant of its lipid state and presence of sterols reduces the fluidity in the core of lipid bilayer, thus favouring the liquid ordered phase (less thermal motion in the acyl chains of lipid bilayer but lateral movements in the plane of bilayer takes place) to increase the thickness of lipid leaflet. Sakamoto et al., 1987 reported that the intravenous administration of γ-oryzanol and cycloartenol ferulic acid ester 10mg/kg for 6 days significantly inhibited the increase in lipids and atherogenic indices and accelerated their excretion from blood.

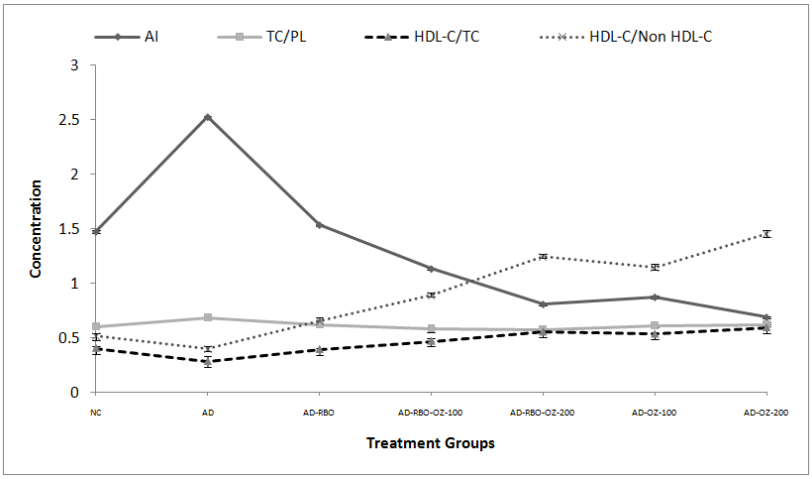

Figure 2: Effects of RBO and OZ Enriched Atherogenic Diets on Serum Indices in Hyperlipidemic Rats (Values (Mean± SEM) of 6 Rats in Each Group)

Hepatic Lipid Profile

High cholesterol feeding has been used to elevate hepatic cholesterol levels in experimental animals to assess hypercholesterolemia and its associated metabolic disturbances in animal models (Mansour et al., 2009; Hozumi et al., 1995). In the present study elevated levels of hepatic lipids (TC, TG, PL) in animals fed on atherogenic diets are consistent with the earlier studies owing to the increased deposition of lipids levels in hepatic tissue. The lipid lowering effect of RBO and OZ enriched diets in experimental animals may be due to inhibition of denovo synthesis, intestinal absorption, or increased catabolism of cholesterol and fatty acids (Ahmad et al., 2014; Mansour et al., 2009) (Table 2)

Hepatic HMG Co. A Reductase Activity

Liver is the main seat for cholesterol metabolism and is regulated by maintaining an equilibrium between its synthesis; its absorption (from intestinal tract) and clearance from enterohepatic circulation through bile excretion. HMG CoA reductase is the rate limiting enzyme in cholesterol biosynthesis (Goldstein and Brown, 1990). Assay of the enzymatic activity is used as a marker to confirm the reduction in hepatic cholesterol is mediated by its inhibition. The HMG-CoA/mevalonate ratio is inversely proportional to the enzyme activity. The ratio decreased significantly in AD group (4.4±0.87) as compared to normal control ones (9.5±1.22). Animals treated with RBO and OZ had a negative impact on the enzyme activity and the maximal increase in the ratio was almost parallel in rats reared on AD-OZ-200 (10.4±1.49) and AD-RBO-OZ-200 (10.3±1.07) diets (Table 2). The suppression of enzymatic activity in the treated animals with a simultaneous decline in cholesterol levels and significant increase in bile acids confirms the interconversion of endogenous cholesterol to bile acids for excretion which corresponds to earlier studies (Wang et al., 2014; Visavadiya and

Narasimhacharya, 2009).

Table 2: Effect of RBO and OZ Enriched Atherogenic Diets on Hepatic Lipids, HMG Co. A Reductase activity and Bile Acids in Hyperlipidemic Rats

Treatment	Triglycerides	Total Cholesterol	Phospholipids	HMG Co.A Reductase/ Mevalonate Ratio	Bile Acids
	(mg/100g)	(mg/100g)	(mg/100g)		(mg/g)
NC	429.8±11.23	373.8±7.82	1800.2±72.4	9.5±1.22	3.5±0.12
AD	505.8±8.52 [a]	420.7±5.65 [a]	2198.5±68.7 [a]	4.4±0.87 [a]	7.7±0.15 [a]
AD-RBO	417.4±9.13 [b]	372.3±7.31 [b]	1899.2±87.8 [b]	5.1±0.14 [b]	7.2±0.17 [b]
AD-RBO-OZ-100	411.2±13.25 [b]	364.8±8.61 [b]	1881.3±76.8 [b]	8.3±1.21 [b]	7.9±0.11 [b]
AD-RBO-OZ-200	402.3±8.17 [b]	355.3±9.61 [b]	1873.2±88.9 [b]	10.3±1.07 [b]	8.8±0.12 [b]
AD-OZ-100	412.8±6.29 [b]	348.2±8.42 [b]	1880.2±98.7 [b]	10.1±2.13 [b]	8.3±0.14 [b]
AD-OZ-200	409.2±7.59 [b]	343.2±8.48 [b]	1871.8±98.3 [b]	10.4±1.49 [b]	8.8±0.17 [b]

Values are Mean ± SEMof 6 rats in each group
Group NC is compared with Groups AD
Other Treatment groups are compared with AD
[a]p≤0.05 : Significantly different from NC
[b]p≤0.05 : Significantly different from AD
NS: Non Significant

Fecal Weight, Neutral Sterol, Cholesterol and Bile Acid Content

The fecal matter, neutral sterols, cholesterol and bile acid excretion increased remarkably after supplementation of RBO and OZ enriched diets to experimental animals as evident from Table 3. The decreased hepatic lipid profile of the treated rats with concomitant increased excretion of fecal neutral sterols, cholesterol and bile acid is indicative of reduced intestinal absorption and increased conversion of excess cholesterol to bile acids in the enterohepatic circulation thus regulating cholesterol metabolism. Our results are in accordance with previous studies whereby different animal models supplemented with RBO and its unsaponifiable matter significantly increased the faecal excretion of acids and neutral sterols (Shinomiya et al., 1983; Sakamato et al., 1987; Seetharamaiah and Chandrasekhra, 1989). Eventually the bioactive compounds in plants, fruits and vegetables have shown a similar effect (Visavadiya and Narasimhacharya, 2009; Garcia-diez et al., 1996).

Table 3: Effect of RBO and OZ Enriched Atherogenic Diets on Fecal Weight, Neutral Sterol, Cholesterol and Bile Acid Content in Hyperlipidemic Rats

Treatment	Faecal Weight	Neutral Sterols	Cholesterol	Bile Acids
	(g/day)	(mg/ g faeces)	(mg/g faeces)	(µg/ g faeces)
NC	8.7±1.27	4.83±1.11	0.45±0.01	0.73±0.01
AD	6.3±1.45[a]	3.17±0.98 [a]	4.3±0.24 [a]	0.45±0.02 [a]
AD-RBO	7.5±1.83 [b]	11.3±1.24 [b]	4.6±0.17[NS]	2.2±0.89 [b]
AD-RBO-OZ-100	7.5±1.57 [b]	13.3±1.54 [b]	4.9±0.82 [b]	2.3±0.94 [b]
AD-RBO-OZ-200	8.2±2.14 [b]	14.8±1.76 [b]	5.3±0.54 [b]	2.3±1.12 [b]
AD-OZ-100	7.7±1.71 [b]	13.9±1.84 [b]	5.1±0.74 [b]	2.4±0.87 [b]
AD-OZ-200	8.9+1.43 [b]	15.8±1.57 [b]	5.7±0.41 [b]	2.8±0.57 [b]

Values are Mean ± SEMof 6 rats in each group
Group NC is compared with Groups AD
Other Treatment groups are compared with AD
[a]p≤0.05 : Significantly different from NC
[b]p≤0.05 : Significantly different from AD
NS: Non Significant

Oxidative Stress Biomarkers

Free radical mediated oxidative stress implicated in the genesis and progression of cardiovascular diseases (CVD). The imbalance created between production of free radicals (FR); reactive oxygen species (ROS) and the effectiveness of antioxidant defense system eventually leads to oxidative stress. The endogenous defense system tends to counter the activity of free radicals thus protecting the cellular functions and preventing the onset of degenerative diseases.

High fat and high cholesterol feeding in animals result in hyperlipidemia induced oxidative stress which in turn stimulates lipid peroxidation (LPO) consistent with many other reports (Chauhan et al., 2015; Chauhan et al., 2010).

Chronic hyperlipidemia resulted in significant increase in TBARS levels with simultaneous decrease in GSH content in serum, hepatic and brain tissue in AD fed group as compared to the normal control. Treatment with RBO and OZ during the feeding regime reversed the effect and restored the levels. Table 4 shows that there was an appreciable improvement in TBARS levels and GSH activity among all treatment groups however the effect was more intensified in the group reared on AD-OZ-200 (27.8±2.19 nmol ofTBARS/ml; 0.74±0.02 nM ofTBARS/mg protein; and 3.7±1.32 nM ofTBARS/mg protein) and (43.7±2.87 mM/100ml; 372.9±7.21 mM/100g; 26.8±2.41mM/100g) in serum, hepatic and brain tissue respectively. Younes and Siegers, (1980) demonstrated that glutathione depletion per se leads to an increased lipid peroxidation possibly due to the its lack as a part of cellular defense system against endogenous toxic intermediates.

Antioxidant enzymes (superoxide dismutase (SOD); catalase (CAT); Glutathione peroxidase (GSHPx); glutathione- S- transferase (GST)) showed a significant decrease ($p \leq 0.05$) in activities in serum; hepatic and brain tissues of animals fed on atherogenic diet (Table 4). Co-administration of RBO and OZ in different doses significantly restored the reduced activities, resulting in enhancement in the enzymatic activities. This is in agreement with other studies whereby the bioactive components with antioxidative potential prevents the formation of unwanted free radicals and maintain cellular and tissue integrity (Aboonabi et al., 2014; Chauhan et al., 2012; Sharma and Garg , 2009).

Protein carbonyl (PCO) is an indicator of oxidative deterioration of proteins. PCO content likewise increased in animals fed on high fat high cholesterol diets indicating diet induced hyperlipidemia resulted in increased oxidative stress load. Our results demonstrate that RBO and OZ supplemented groups showed a marked decline ($p \leq 0.05$) in PCO content (Table 4). Proteins act as a carrier for transporting important metabolites, nutrients and other substances. They are targeted by free radicals, resulting in conformation changes, oxidative modification thereby leading to loss of normal physiological functions and activities. The results are in accordance with previous study which demonstrated that aqueous enzymatic extract from rice bran is effective in strengthening antioxidant defense system; preventing peroxidative damage and improving the protein carbonyl content (Wang et al., 2014).

Table 4: Effect of RBO and OZ Enriched Atherogenic Diets on

Oxidative Stress Biomarkers in Hyperlipidemic Rats

	NC	AD	AD-RBO	AD-RBO-OZ-100	AD-RBO-OZ-200	AD-OZ-100	AD-OZ-200
Serum							
TBARS (nmol of TBARS/ml)	24.4±1.25	47.8±2.15 [a]	41.6±2.54 [b]	37.2±3.12 [b]	33.4±2.71 [b]	35.2±2.57 [b]	27.8±2.19 [b]
GSH (mM/100ml)	44.3±2.87	28.2±2.57 [a]	35.2±2.96 [b]	38.6±2.41 [b]	42.8±3.21 [b]	41.7±3.41 [b]	43.7±2.87 [b]
SOD (Units/ 100ml)	295.6±11.2	93.1±7.83 [a]	145.8±5.84 [b]	168.4±7.87 [b]	219.5±4.87 [b]	189.6±8.67 [b]	211.4±7.54 [b]
CAT (value x10-3 units/100ml)	12.3±1.12	8.4±1.10 [a]	9.1±1.57 [NS]	9.3±1.34 [b]	11.8±2.10 [b]	10.8±2.14 [b]	12.1±1.87 [b]
GSHPx(GSH utilized per minute/ 100ml)	720.4±8.95	613.2±12.23 [a]	679.4±11.45 [b]	692.6±11.78 [b]	702.5±8.98 [b]	698.4±9.78 [b]	715.8±7.84 [b]
GST (nM/minute/mg protein)	41.2±1.37	34.1±2.41 [a]	35.7±2.63 [NS]	35.9±3.12 [NS]	40.2±2.48 [b]	39.8±2.57 [b]	42.4±3.45 [b]
Protein Carbonyl (nmol/mg protein)	0.061±0.01	0.087±0.01 [a]	0.075±0.02 [b]	0.071±0.05 [b]	0.065±0.01 [b]	0.068±0.05 [b]	0.061±0.03 [b]
Hepatic Tissue							
TBARS (nM of TBARS/mg protein)	0.76±0.01	0.87±0.04 [a]	0.85±0.03 [NS]	0.81±0.02 [NS]	0.75±0.07 [b]	0.78±0.01 [b]	0.74±0.02 [b]
GSH (mM/100g)	384.5±8.74	273.1±9.87 [a]	333.4±9.67 [b]	345.4±8.54 [b]	357.2±8.19 [b]	359.6±9.45 [b]	372.9±7.21 [b]
SOD (Units/ mg protein)	2.8±0.08	1.7±0.11 [a]	2.1±0.14 [NS]	2.3±0.17 [b]	2.7±0.15 [b]	2.7±0.21 [b]	2.9±0.45 [b]
CAT (value x10-3 unit/mg protein)	56.9±4.25	46.8±3.15 [a]	51.8±4.87 [b]	51.4±3.24 [b]	53.4±3.89 [b]	53.5±4.21 [b]	55.8±6.94 [b]
GSHPx(GSH utilized per minute/ mg protein)	8.6±1.21	6.3±2.21 [a]	8.4±2.58 [b]	8.1±1.27 [b]	8.7±1.64 [b]	8.2±1.23 [b]	8.5±1.29 [b]
GST (nM/minute/mg protein	522±17.45	287±18.5 [a]	338.8±21.84 [b]	361.7±17.87 [b]	447.2±17.91 [b]	483.2±21.34 [b]	512.1±11.21 [b]
Protein Carbonyl (nmol/mg protein)	1.8±0.01	3.4±1.04 [a]	2.6±1.23 [b]	2.5±1.02 [b]	2.1±1.21 [b]	2.3±1.47 [b]	1.9±0.07 [b]
Brain Tissue							
TBARS (nM of TBARS/mg protein)	3.4±1.01	6.6±1.23 [a]	5.3±1.27 [b]	5.5±1.64 [b]	4.5±1.21 [b]	4.2±1.71 [b]	3.7±1.32 [b]
GSH (mM/100g)	27.4±2.10	17.3±2.15 [a]	20.1±2.23 [b]	22.1±2.17 [b]	24.6±2.24 [b]	24.1±2.37 [b]	26.8±2.41 [b]
SOD (Units/ mg protein)	53.7±3.65	44.2±3.18 [a]	49.5±2.98 [NS]	49.4±3.75 [b]	51.6±3.84 [b]	49.8±4.21 [NS]	52.3±3.56 [b]
CAT (value x10-3 unit/mg protein)	12.2±1.61	5.7±1.34 [a]	6.3±1.27 [NS]	6.5±1.54 [b]	9.2±2.14 [b]	8.7±1.36 [b]	11.4±2.15 [b]
GSHPx(GSH utilized per minute/ mg protein)	65.4±3.21	56.7±3.98 [a]	59.7±3.49 [NS]	59.4±3.85 [NS]	61.2±3.96 [b]	60.4±3.78 [b]	63.7±3.81 [b]
GST (nM/minute/mg protein	157.2±3.14	125.8±3.17 [a]	127.8±2.14 [NS]	134.4±2.17 [b]	142.6±3.45 [b]	141.0±2.86 [b]	147.4±3.24 [b]
Protein Carbonyl (nmol/mg protein)	2.4±0.15	3.8±0.75 [a]	3.2±0.18 [b]	3.1±0.67 [b]	2.6±0.35 [b]	3.1±0.27 [b]	2.6±0.84 [b]

Liver Function Tests

Figure3. Depicts the enzymatic activities of serum transaminases (ALT and AST); alkaline phosphatase (AP) and γ- glutamyl transferase (GGT) as indicators of hepatic malfunctioning. The atherogenic diet fed rats showed an upsurge in the enzymatic activities as compared to the normal fat fed ones indicating increased oxidative stress in the liver associated with hyperlipidemia. Incorporation of RBO and OZ to AD lowered the activities, although the decrease in ALT activity was not statistically significant. A remarkable decline was observed in AST activities resulting in the levels lower than the normal (62.3±3.17U/ml) in the group reared on AD-OZ-200 (60.1±3.71U/ml). The resultant decrease in the enzyme activities owing to supplementation of RBO and OZ to AD indicate their protective potential against liver damage due to membrane disintegrity and distress in hyperlipidemic rats. Results are consistent with previous studies (Chauhan et al., 2015; Ramesh and Pugalendi, 2006)

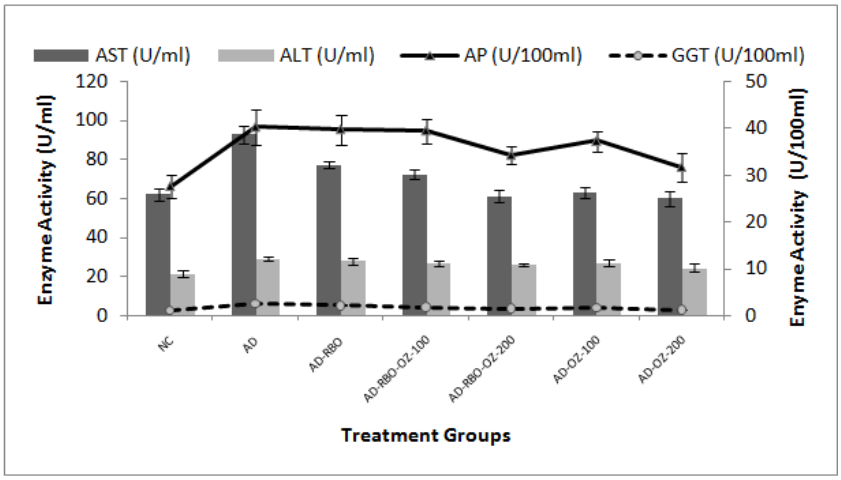

Figure 3: Effect of RBO and OZ Enriched Atherogenic Diets on Liver Function Tests in Hyperlipidemic Rats (Values (Mean± SEM) of 6 rats in each group)

Renal Function Tests

Plasma urea nitrogen; creatinine and uric acid are significant markers for kidney dysfunction. The markers significantly increased in AD group as compared to the normal control, indicating atherogenic diet induced hyperlipidemia with resultant oxidative stress impaired the normal physiological functions of the nephrotic tissue. Feeding of enriched diets to experimental animals showed a decreasing trend in all the parameters, however the decrease in uric acid was not significant ($p \geq 0.05$) and plasma creatinine levels and urea nitrogen levels showed marked improvement ($p \leq 0.05$) in AD-OZ-200 (0.5±0.002mg/dl and 15.3±1.17 mg/dl) group followed by AD-RBO-OZ-200 (0.61±0.01mg/dl and 18.3±1.87mg/dl) respectively. The nephroprotective effect of RBO and OZ is probably by virtue of antioxidative potential of the bioactive compounds. The results are in agreement with earlier studies whereby the renal functions showed improvement upon supplementing certain herbs rich in antioxidants to hyperlipidemic rats (Ahmad et al., 2014; Halaby et al., 2013)

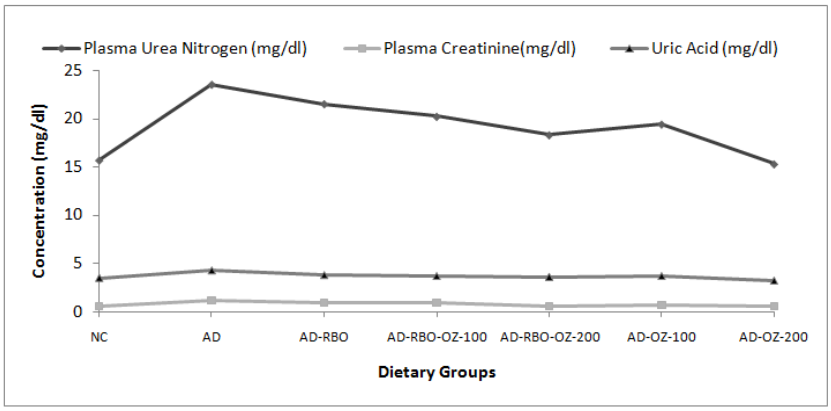

Figure 4: Effect of RBO and OZ Enriched Atherogenic Diets on Renal Function Tests in Hyperlipidemic Rats (Values (Mean± SEM) of 6 Rats in Each Group)

CONCLUSIONS

The antioxidative potential elucidated by RBO and OZ is probably due to their ability to activate antioxidant enzymes that catalyze the reduction of oxidants. Rice bran oil and oryzanol effectively regulates lipid lipoprotein metabolism and its antioxygenic potential protects against oxidative stress thus can be used as therapeutic weapon in the management of dyslipidemia and associated cardiovascular diseases.

ACKNOWLEDGEMENTS

The authors like to acknowledge University Grant Commission for financial support and Dr. Ajit Kumar, Vice Chancellor, NIF TEM for his unflagging support and valuable suggestions

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